polymerase, the solid line represents the DNA which is transcribed from plasmids which have been digested with MboII. The white box represents sequences encoding the recognition sites for MboII, EcoRI and PstI, in that order. Sites of cleavage by restriction endonucleases are indicated. Beneath the diagram, the entire sequences of RNAs which result from synthesis by T7 RNA polymerase from MboII-digested plasmid are given. The V-wt RNA (SEQ ID NO: 49) has the identical 5' and 3' termini as found in RNA segment 8 of influenza A viruses, separated by 16 "spacer" nucleotides. The RNA, M-wt (SEQ ID NO: 50), represents the exact opposite stand, or "message-sense", of V-wt (SEQ ID NO: 49). Restriction endonuclease sites for DraI, EcoRI, PstI and SmaI are indicated. T7 transcripts of plasmids cleaved by these enzymes result in, respectively, 32, 58, 66 and 91 nucleotide long RNAs. The sequences of V-d5' RNA (SEQ ID NO: 51) are indicated. The plasmid design is essentially the same as that used for the V-wt RNA (SEQ ID NO: 49) except for the minor changes in the "spacer" sequence. The point mutants of V-d5' RNAs which were studied are indicated in Table I.--

On page 18, please replace the paragraph beginning, "FIG. 11(A)." with the following paragraph:

--FIG. 11.(A). Diagrammatic representation of relevant portions of pIVCAT1. The various domains are labeled and are, from left to right; a truncated T7 promoter; the 5' nontranslated end of influenza A/PR/8/34 virus segment 8 (22 nucleotides); 8 nucleotides of linker sequence; the entire CAT gene coding region (660 nucleotides) the entire 3' nontranslated end of influenza A/PR/8/34 virus segment 8 (26 nucleotides); and linker sequence containing the HgaI restriction enzyme site. Relevant restriction enzyme sites and start and stop sites for the CAT gene are indicated. (B) The 716 base RNA product obtained following HgaI digestion and transcription of pIVACAT1 by T7 RNA polymerase. Influenza viral sequences are indicated by bold letters, CAT gene sequences by plain letters, and linker sequences by italics. The triplets -- in antisense orientation --representing the initiation and termination codons of the CAT gene are indicated by arrow and underline, respectively (SEQ ID NOS: 52 and 58).--

On page 20, please replace the paragraph beginning, "FIG. 16." with the following paragraph:

--FIG. 16. Diagram of relevant portions of the neuraminidase (NA) gene contained in plasmids used for transfection experiments. The pUC19 derived plasmid pT3NAv contains the influenza A/WSN/33 virus NA gene and a truncated promoter specifically recognized by bacteriophage T3 RNA polymerase. The T3 promoter used is truncated such that the initial transcribed nucleotide (an adenine) corresponds to the 5' adenine of the WSN NA gene. At the 3' end of the cDNA copy of the NA gene, a Ksp632I restriction enzyme site was inserted such that the cleavage site occurs directly after the 3' end of the NA gene sequence (SEQ ID NO: 55). A 1409 nucleotide long transcript was obtained following Ksp632I digestion and transcription by T3 RNA polymerase of PT3NAv (as described in Section 8.1, infra). The 15 5' terminal nucleotides, the 52 nucleotides corresponding to the region between the restriction endonuclease sites NcoI and PstI and the 12 3' terminal nucleotides are shown (SEQ ID NOS:53, 59 and 55). The transcript of pT3NAv mut 1 (SEQ ID NO: 54) is identical to that of pT3NAv (SEQ ID NO: 53) except for a single deletion, eleven nucleotides downstream from the 5' end of the wild type RNA (SEQ ID NO: 53). The transcript of the pT3NAv mut 2 is identical to that of pT3NAv except for 5 mutations located in the central region (indicated by underline) (SEQ ID NO: 61). These five mutations do not change the amino acid sequence in the open reading frame of the gene. The serine codon UCC at position 887-889 (plus sense RNA) was replaced with the serine codon AGU in the same frame. The numbering of nucleotides follows Hiti et al., 1982, J. Virol. 41:730-734.--

On page 22, please replace the paragraph beginning, "FIG. 18." with the following paragraph:

--FIG. 18. Sequence analysis of RNA obtained from rescued influenza virus containing five site-specific mutations. Following infection with the WSN-HK helper virus, MDBK cells were RNP-transfected with T3NAv mut 2 RNA which was obtained by transcription from pT3NAv mut 2. Following overnight incubation in the presence of 28 μg/ml plasminogen, medium was used for propagation and plaquing on MDBK cells in the absence of protease. Virus from plaques was then amplified

and RNA was obtained following phenol-extraction of purified virus. Rescue of the mutant NA gene into virus particles was verified through direct RNA sequencing using 5'-TACGAGGAAATGTTCCTGTTA-3' (SEQ ID NO: 1) as primer (corresponding to position 800-819; Hiti et al., J. Virol. 41:730-734) and reverse transcriptase (Yamashita et al., 1988, Virol. 163:112-122). Sequences shown correspond to position 878-930 in the NA gene (SEQ ID NO: 56) (Hiti et al., J. Virol. 41:730-734). The arrows and the underlined nucleotides indicate the changes in the mutant RNA compared to the wild type RNA. Left: Control RNA obtained from influenza A/WSN/33 virus. Right: RNA of mutant virus rescued from MDBK cells which were RNP-transfected with T3NAv mut 2 RNA and infected with helper virus WSN-HK.--

On page 35, please replace the paragraph beginning, "Depending on the integrity of the foreign gene product and the purpose of the construction" with the following paragraph:

--Depending on the integrity of the foreign gene product and the purpose of the construction, it may be desirable to construct hybrid sequences that will direct the expression of fusion proteins. For example, the four influenza virus proteins, PB2, PB1, PA or NP are polymerase proteins which are directed to the nucleus of the infected cell through specific sequences present in the protein. For the NP this amino acid sequence has been found to be (single letter code) QLVWMACNSAAFEDLRVLS (SEQ ID NO: 2) (Davey et al., 1985, Cell 40:667-675) (seq. Therefore, if it is desired to direct the foreign gene product to the nucleus (if by itself it would not ordinarily do so) the hybrid protein should be engineered to contain a domain which directs it there. This domain could be of influenza viral origin, but not necessarily so. Hybrid proteins can also be made from non-viral sources, as long as they contain the necessary sequences for replication by influenza virus (3' untranslated region, etc.).--

On page 35, please replace the paragraph beginning, "As another example" with the following paragraph:

--As another example, certain antigenic regions of the viral gene products may be substituted with foreign sequences. Townsend et al., (1985, Cell 42:475-482),

identified an epitope within the NP molecule which is able to elicit a vigorous CTL (cytotoxic T cell) response. This epitope spans residues 147-161 of the NP protein and consists of the amino acids TYQRTRQLVRLTGMDP (SEQ ID NO: 3). Substituting a short foreign epitope in place of this NP squence may elicit a strong cellular immune response against the intact foreign antigen. Conversely, expression of a foreign gene product containing this 15 amino acid region may also help induce a strong cellular immune response against the foreign protein.--

On pages 54- 56, please replace the paragraph beginning, "The plasmid design is indicated in FIG. 2" with the following paragraph:

--The plasmid design is indicated in FIG. 2. Insert DNA for the pV-wt plasmid was prepared using an Applied Biosystems DNA synthesizer. The "top" strand was 5'-
GAAGCTTAATACGACTCACTATAAGTAGAAACAAGGGTGTTTTTCATATC ATTTAAACTTC ACCCTGCTTTTGCTGAATTCATTCTTCTGCAGG-3' (SEQ ID NO: 4). The "bottom" strand was synthesized by primer-extension with 5'-CCTGCAGAAGAATGA-3' (SEQ ID NO: 57) as primer. The 95 bp DNA was digested with HindIII and PstI and purified by extraction with phenol/chloroform, ethanol precipitation, and passage over a NACS-prepack ion exchange column (Bethesda Research Laboratories). This DNA was ligated into pUC-19 which had been digested with HindIII and PstI and then used to transform E. coli strain DH5-α which had been made competent using standard protocols. Bacteria were spread on agar plates containing X-gal and IPTG, and blue colonies were found to have the plasmid containing the predicted insert since the small insert conserved the lacZ reading frame and did not contain a termination codon. The pM-wt plasmid was prepared by a similar strategy except that both strands were chemically synthesized with the upper strand having the sequence
5'-
GAAGCTTAATACGACTCACTATAAGCAAAAGCAGGGTGAAGTTTAAATGA TAT-GAAAAAACACCCTTGTTTCTACTGAATTCATTCTTCTGCAGG-3' (SEQ ID NO: 5).

The pV-d5' plasmid (FIG. 2) was prepared using the oligonucleotides 5'-AGCTTAATACGACTCACTATAAGATCTATTAAACT-TCACCCTGCTTTTGCTGAATTCATTCTTCTGCA-3' (SEQ ID NO: 6) and 5'-GAAGAATGAAT-TCAGCAAAAGCAGGGTGAAGTTTAATAGATCTTATAGTGAGTCGTATTA-3' (SEQ ID NO: 7). The DNAs were annealed and ligated into the HindIII/PstI digested pUC-19 and white colonies were found to contain the correct plasmid because this insert resulted in a frameshift in the lacZ gene. The point mutants were isolated following digestion of pV-d5' with BglII and PstI and ligation of the linearized plasmid with a single stranded oligonucleotide of mixed composition. Since BglII laves a 5' extension and PstI a 3' extension, a single oligonucleotide was all that was necessary for ligation of insert. The host cell was then able to repair gaps caused by the lack of a complementary oligonucleotide. Oligonucleotides were designed to repair the frameshift in the lacZ gene so that bacteria which contained mutant plasmids were selected by their blue color.--

On page 56, please replace the paragraph beginning, "Plasmid pHgaNS" with the following paragraph:

--Plasmid pHgaNS, which was used to prepare an RNA identical to segment 8 of A/WSN/33, was prepared using the primers 5'-CCGAATTCTTAATACGACTCACTATAAGTAGAAACAAGGGTG-3' (SEQ ID NO: 8) and 5'-CCTCTAGACGCTCGAGAGCAAAAGCAGGTG-3' (SEQ ID NO: 9) in a polymerase chain reaction off a cDNA clone. The product was then cloned into the XbaI/EcoRI window of pUC19.--

On page 67, please replace the table titled, "**TABLE II**" with the following table:

TABLE II

QUANTITATIVE COMPARISON OF THE EFFECT OF POINT MUTATIONS IN THE PROMOTER SEQUENCE*

RNA Template	3' sequence	Level of Synthesis	SEQ ID NO.
V-d5'	CACCCUGCUUUUGCU-OH	1	10
V-A3	CACCCUGCUUUUACU-OH	0.4	11
V-C5	CACCCUGCUUCUGCU-OH	1.0	12
V-d$U_{25}U_8$	CACCCUGUUUUUGCU-OH	1.0	13
V-U_8A_3	CACCCUGUUUUUACU-OH	0.08	15
V-U_8C_5	CACCCUGUUUCUGCU-OH	0.3	16
V-iU_{10}	CACCCUUGCUUUUGCU-OH	0.7	14
V-i$U_{10}A_3$	CACCCUUGCUUUUACU-OH	0.06	17
V-i$U_{10}U_8A_3$	CACCCUUGUUUUUACU-OH	0.2	18
V-i$U_{10}U_8C_5A_3$	CACCCUUGUUUCUACU-OH	0.2	19

* Sequences of V-wt, M-wt and V-d5' are shown in FIG. 2. All other RNAs are identical to V-d5' except for the indicated positions. The subscripted number indicates the distance from the 3' end of a change, and d and i refer to deleted or inserted nucleotides.

On page 72, please replace the paragraph beginning, "In order to get the flanking sequences of the NS RNA fused to the coding sequence of the CAT gene" with the following paragraph:

--In order to get the flanking sequences of the NS RNA fused to the coding sequence of the CAT gene, the following strategy was used. Two suitable internal restriction sites were selected, close to the start and stop codon of the CAT gene, that would allow the replacement of the sequences flanking the CAT gene in the pCM7 plasmid with the 3'- and 5'-NS RNA sequences. At the 5' and, a SfaNI site was chosen, (which generates a cut 57 nt from the ATG) and at the 3'- end a ScaI site which generates a cut 28 nt from the end of the gene (stop codon included). Next, four synthetic oligonucleotides were made using an Applied Biosystems DNA synthesizer, to generate two double-stranded DNA fragments with correct overhangs for cloning. Around the start codon these oligonucleotides formed a piece of DNA containing a XbaI overhang followed by a HgaI site and a PstI site, the 3'-(viral-sense) NS sequence immediately followed by the CAT sequence from start codon up to the SfaNI overhang (underscored). In addition a silent mutation was incorporated to generate an AccI site closer to the start codon to permit future modifications.

Xba I

Hga I Pst I Acc I

3'tgcgggacgtcgttttcgtcccactgtttctgtattacctctttttttagtg

SfaN I

acccatatggtggcaactatatagggttagcgtagcatttcttg- 5' (SEQ ID NO: 21) oligo1

Xba I

Hga I Pst I Acc I

5'-ctagacgccctgcagcaaaagcagggtgacaaagacataatggagaaaaaaatcac

SfaN I

tgggtataccaccgttgatatatcccaatcgcatcgtaaa- 3' (SEQ ID NO: 62) oligo2

Around the stop codon the two other oligonucleotides generated a piece of DNA as follows: a blunt-ended ScaI site, the CAT sequence from this site up to and including the stop codon (underlined) followed by a BglII site and a Xba I overhang.

Sca I Bgl II

5'-actgcgatgagtggcagggcgggcgtaatagat- 3' (SEQ ID NO: 22) oligo3

3'-tgacgctactcaccgtcccgccccgcattatctagatc- 5' (SEQ ID NO: 25) oligo4

XbaI --

On page 75, please replace the paragraph beginning, "pIVACAT1 was constructed in the following way:" with the following paragraph:

--pIVACAT1 was constructed in the following way: In order to obtain the correct 5'- end in pIVACAT1, the EcoRI-ScaI fragment of the CAT gene derived from plasmid pCM7 (Pharmacia) was ligated to a DNA fragment formed by two synthetic oligonucleotides. The sequence of these oligonucleotides are: 5'-ACTGCGATGAGTGGCAGGGCGGGGCGTAATA-GAT- 3' (top strand) (SEQ ID NO: 22), and 5'-CTAGATCTATTACGCCCCGCCCTGCCACTCATCGCAGT- 3' (bottom strand) (SEQ ID NO: 23). For the 3'- end of the insert in pIVACAT1 the SfaN 1-EcoRI fragment of the CAT gene was ligated to a DNA fragment made up of the synthetic oligonucleotdies:

5'-CTAGACGCCCTGCAGCAAAAGCAGGGTGAC-
AAAGACATAATGGAGAAAAAAATCACTGGGTATACCACCGTTGATATAT
CCCAATCG-
CATCGTAAA- 3' (top strand) (SEQ ID NO: 26), and
5'-GTTCTTTACGATGCGATTGGGAT-
ATATCAACGGTGGTATACCCAGTGATTTTTTTCTCCATTATGTCTTTGTCAC
CCTGCT-
TTTGCTGCAGGGCGT- 3' (bottom strand) (SEQ ID NO: 27). Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer. These 5' and 3' constructs were ligated into pUC19 shuttle vectors digested with XbaI and EcoRI, grown up, cut out with EcoRI/BglII (5' region) and XbaI/EcoRI (3' region) and ligated into BglII/XbaI cut pPHV. The latter plasmid is similar to pV-WT described in Section 6, supra, except that it contains a BglII site which separates the noncoding terminal sequences of the influenza A virus NS RNA segment. The final clone pIVACATl (FIG. l) was grown up and the DNA was partially sequenced starting from the flanking pUC sequences and reaching into the CAT gene. No changes were found as compared to the expected sequences with the exception of a silent G to A transition in the CAT gene at position 106 relative to the start of the IVACATl RNA.--

On page 84, please replace the paragraph beginning, "The pT3NAv, pT3NAv mut 1 and pT3NAv mut 2 plasmids were constructed by PCR-directed mutagenesis" with the following paragraph:

--The pT3NAv, pT3NAv mut 1 and pT3NAv mut 2 plasmids were constructed by PCR-directed mutagenesis using a cloned copy of the WSN NA gene, which was obtained following standard procedures (Buonagurio et al., 1986, Science 232:980-982). To construct pT3NAv, the following primers were used: 5'-CGGAATTCTCTTCGAGCGAAAGCAGGAGTT-3' (SEQ ID NO: 28) and 5'-CCAAGCTTATTAACCCTCACTAAAAGTAGAAACAAGGAGTTT-3' (SEQ ID NO: 63). After 35 cycles in a thermal cycler (Coy Lab products, MI), the PCR product was digested with EcoRI and HindIII and cloned into pUC19. Plasmid

pT3NAv mut 1 was constructed in a similar fashion except that the sequence of the primer was altered (FIG. 16). Plasmid pT3NAv mut 2 was constructed by cassette mutagenesis through the digestion of pT3NAv with PstI and NcoI and religation in the presence of the synthetic oligonucleotides - 5'-CATGGGTGAGTTTCGACCAAAATCTAGATTAT-AAAATAGGATACATATGCA-3' (SEQ ID NO: 29) and 5'-AATGTATCCTATTTTATAATCTAGATTTTGGTCGAAACTCACC-3' (SEQ ID NO: 31). Oligonucleotides were synthesized on an applied Biosystems DNA synthesizer. The final clones pT3NAv, pT3NAv mut 1 and pT3NAv mut 2 were grown up and the DNAs were partially sequenced starting from the flanking pUC19 sequences and reaching into the coding sequences of the NA gene. The mutations in pT3NAv mut 2 were also confirmed by sequencing.--

On pages 94 - 97, please replace the paragraph beginning, "Construction of plasmids" with the following paragraph:

--Construction of plasmids. Plasmids were constructed by standard techniques (Maniatis, T., 1982, Molecular Cloning: A Press Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). pT3NACAT(wt) contains the CAT gene in negative polarity flanked by the 3' and 5' noncoding regions of the WSN NA gene, under the transcriptional control of a truncated T3 promoter. pT3NA/BIP was constructed as follows: first, a PCR product was obtained using the oligonucleotides 5'-GGCCACTAGTAGGTCGACGCCGGC-3' (SEQ ID NO: 32) and 5'-GCGCTGGCCATCTTGCCAGCCA-3' (SEQ ID NO: 33) as primers, and a plasmid containing the 5' noncoding region of the BiP gene as template. This PCR product was digested with restriction enzymes *Msci and SpeI* and cloned into *Msci and XbaI* digested pT3NA/EMC. The resulting plasmid, pT3NA/BIP contains the ORF of the NA followed by the IRES sequences of the BiP gene (nucleotide positions 372 to 592 of the GenBank data base entry HUMGRP78). pT3NA/BIP-CAT contains, in addition, the CAT ORF following the BiP-IRES-derived sequences. pT3NA/BIP-CAT was constructed by inserting into Msci digested pT3NA/BIP the PCR product

which was obtained by using the primers 5'-AGAAAAAAATCACTGGG-3' (SEQ ID NO: 34) and 5'-TTACGCCCCGCCCTGCC-3' (SEQ ID NO: 35) and template pIVACAT1/S (Piccone, M.E. et al., 1993, Virus Res. 28:99-112). A fragment of approximately 920 nt derived from the NA ORF was deleted from pT3NA/BIP-CAT by digestion with *PpuM1 and Spe1,* trimming and religation of the plasmid. The resulting plasmid was called pT3delNA/BIP-CAT. To construct pT3BIP-NA, the PCR product obtained by using the primers 5'-GCGCATCGAT-AGGTCGACGCCGG-3' (SEQ ID NO: 36) and 5'-GGCCATCGATCCAATGGTTATTATTTCTGGTTTGGA-TTCATCTTGCCAGTTGGG-3' (SEQ ID NO: 37) and a plasmid containing the 5' noncoding region of the BiP gene as template was digested with Cla1 and inserted into Cla1 digested pT3NAM1. pT3NAM1 contains the NA gene of WSN virus into which a *Cla1* site has been inserted at nucleotide positions 52-57 by two silent changes. The resulting plasmid, pT3BIP-NA, has the BIP-IRES-derived sequences in front of the NA ORF. To construct pT3GP2/BIP-NA, a PCR product was obtained using oligonucleotides 5'-ATGACTGGATCCGCTAGCATGGCCATCATTTATCTC-ATTCTCCTGTTCACAGCAGTGAGAGGGGACCAGATAGAAGAATCGCAAAACCAGC-3' (L primer) (SEQ ID NO: 38) and 5'-ATGACAGAATTCGTCGACTTATCTATTCACTACAGAAAG-3'(M primer) (SEQ ID NO: 39) as primers and a plasmid containing the DNA copy of the genome of the HIV-1 isolate BH 10 (GenBank data base entry HIVBH102) as template. The PCR product was digested with *BamH1 and EcoR1* and cloned into *BamH1/EcoR1* digested pGEX-2T (Pharmacia). This clone was used as template for the generation of a PCR product with the primers "M" and 5'-GCGCGAAGACGCAGCAAAAGCAGGAGTTTAAGCTAGCATGGCCATC-ATTTATC-3' (SEQ ID NO: 40). The resulting PCR product was digested with *Bbs1 and Sal1,* and ligated into *Bbs1/Sal1* digested pT3BIP-NA. The resulting plasmid, pT3GP2/BIP-NA, has an ORF in front of the BIP-IRES sequences which codes for a gp4l-derived polypeptide, containing 38 aa of the ectodomain of gp4l, the 22 aa of the

transmembrane domain and 2 aa of the cytoplasmic tail of gp4l. This sequence is preceded by the signal peptide (15 aa) and the first 2 aa of the HA of influenza A/Japan/305/57. For the construction of pT3HGP2/BIP-NA, a PCR product containing the sequences encoding the transmembrane and cytoplasmic tail of the HA of WSN virus was obtained using the primers 5'-CGATGGATCCGCTAGCTTGGAATCGATGGGGGTGTATC-3' (SEQ ID NO: 41) and 5'ATCGATGAATTCGTCGACTCAGATGCATATTCTGCAC-3' (SEQ ID NO: 42) and pT3/WSN-HA (Enami, M. and Palese, P., 1991, J. Virol. 65:2711-2713) as template. This PCR product was digested with restriction enzymes *BamH1 and Sal1* and subcloned into *BamH1/Sal1* digested pGEX-2T. A second PCR product was inserted into this subclone between the *BamH1* and *Cla1* restriction sites. The second PCR product was obtained using oligonucleotides "L" and 5'-ATGACTGTCGACCCATGGAAGTCAATCGATGTTATGTTAAACCAATTCCAC-3' (SEQ ID NO: 43) as primers and the plasmid containing the DNA copy of the HIV-1 genome as template. From this plasmid an *Nhe1-Sal1* fragment was recloned into *Nhe1/Sa1* digested pT3GP2/BIP-NA, and the resulting plasmid was called pT3HGP2/BIP-NA. The first ORF of pT3HGP2/BIP-NA codes for a polypeptide which has an ectodomain (39 aa, of which 31 aa are derived from the gp4l protein ectodomain), followed by the transmembrane and cytoplasmic domains of the HA of influenza WSN virus (37 aa). Oligonucleotides were synthesized using an Applied Biosystems DNA synthesizer. The presence of the appropriate sequences in the plasmid DNAs was confirmed by sequencing with a DNA sequencing kit (United States Biochemical Corporation).--

On page 98, please replace the paragraph beginning, "RNA extraction, electrophoresis and sequencing" with the following paragraph:

--RNA extraction, electrophoresis and sequencing. RNAs were extracted from purified viruses as previously described (Luo, G. et al., 1992, J. Virol. 66:4679-4685). Approximately 100 ng of virion RNAs were electrophoresed on a 2.8% polyacrylamide gel containing 7.7 M urea at 150 V for 110 min. The RNA segments

were visualized by silver staining (Section 8, supra). The NA-RNA segment of transfectant viruses was sequenced as follows: first, 100 ng of viron RNAs were used for a reverse transcription reaction using the primer 5'-GCGCGAATTCTCTTCGAGCAAAAGCAGG-3' (SEQ ID NO: 44) (EKFLU, annealing to the last 12 nt at the 3'end of the influenza A virus RNAs), and SuperScript reverse transcriptase (GibcoBRL). The obtained cDNAs were PCR-amplified using the primers EKFLU and 5'-AGAGATGAATTGCCGGTT-3' (SEQ ID NO: 45) (corresponding to nt positions 243-226 of the NA gene). PCR products were cloned into pUC19 (New England Biolabs), and sequenced with a DNA sequencing kit (United States Biochemical Corporation).--

On page 99, please replace the paragraph beginning, "Immunostaining of Infected cells" with the following paragraph:

--Immunostaining of Infected cells. Confluent MDCK monolayers in a 96-well plate were infected with transfectant or wild-type influenza viruses at an MOI ≥ 2. Nine hours postinfection, cells were washed with PBS and fixed with 25 μl of 1 % paraformaldehyde in PBS. Then, cells were incubated with 100 μl of PBS containing 0.1 % BSA for 1 hour, washed with PBS three times, and incubated 1 h with 50 μl of PBS, 0.1 % BSA containing 2 μg/ml of the human monoclonal antibody 2F5. This antibody recognizes the amino acid sequence Glu-Leu-Asp-Lys-Trp-Ala (ELDKWA)(SEQ ID NO: 46) which is present in the ectodomain of gp41 of HIV-1 (Muster, T. et al., 1993, J. Virol. 67:6642-6647). After three PBS washings, 2F5-treated cells were incubated with 50 μl of PBS, 0.1 % BSA, containing a 1:100 dilution of a peroxidase-conjugated goat antibody directed against human immunoglobulins (Boehringer Mannheim). Finally, cells were PBS-washed three times, and stained with a peroxidase substrate (AEC chromogen, Dako Corporation).--

IN THE SEQUENCE LISTING